REMARKS

Amendments

Claim 12 is amended to correct a typographical error, in accordance with the Examiner's suggestion. New claims 25-30 are directed to further aspects of the invention and are supported throughout the disclosure. No new matter is added.

Objection to claim 12

As noted above, claim 12 is amended in accordance with the Examiner's suggestion. Withdrawal of the objection is respectfully requested.

Rejection under 35 USC 103(a) in view of Cimpoia et al., Janes et al., Ferrero et al., and Martinelle et al.

Claims 1-24 are rejected as allegedly being obvious in view of the article by Janes et al. in combination with Cimpoia et al. (WO 00/47759), the article by Ferrero et al., and the article by Martinelle et al. This rejection is traversed.

In the rejection, it is asserted that Janes et al. disclose a process wherein the compound 2-(*R,S*)-**benzoylmethyl**-4(*S*)-carboxylic acid-1,3-dioxolane methylester is subjected to enzymatic resolution in the presence of the enzyme pig liver esterase. The rejection further suggests that this compound corresponds to a compound of either Formula II or Formula IV of applicants' claims in which R_1/R_{11} is methyl (i.e., a C_{1-12} alkyl) and R_2/R_{12} is **benzoyl** (i.e., a CO-C_{6-12} aryl). This is incorrect.

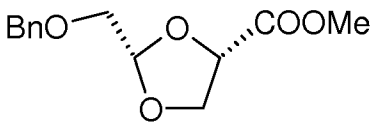
The compounds studied by Janes et al. are **not** the diastereoisomers 2-(*R,S*)-**benzoyl**methyl-4(*S*)-carboxylic acid-1,3-dioxolane methylester. Instead, as can be seen from the Title of the article, the compounds studied are the diastereoisomers 2-(*R,S*)-**benzyl**oxymethyl-4(*S*)-carboxylic acid-1,3-dioxolane methylester. See also page 9028, top left column of Janes et al. In other words, contrary to the assertion in the rejection, in Figure 2 of Janes et al., "Bn" means benzyl, not benzoyl.

It is asserted in the rejection that Janes et al. disclose using *Candida Antarctica* lipase A,

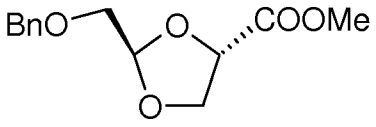
Candida Lypolitica lipase, and *miehei* lipase (i.e., *Mucor miehei* lipase) referring to Table 1. Here again, the enzymatic processes for which data is provided in Table 1 were performed on the substrate 2-(*R,S*)-**benzyloxymethyl**-4(*S*)-carboxylic acid-1,3-dioxolane methylester, not 2-(*R,S*)-**benzoylmethyl**-4(*S*)-carboxylic acid-1,3-dioxolane methylester.

The substrate compound used by Janes et al., 2-(*R,S*)-benzylmethyl-4(*S*)-carboxylic acid-1,3-dioxolane methylester, has only one cleavable carbonyl group which is at the C-4 position. Thus, the disclosure by Janes et al. provides no suggestion of what enzyme would be useful to resolve 2(*S*)/2(*R*) isomers that have two cleavable carbonyl groups at the C-2 and C-4 position. Such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e., discriminating between isomers having the 2(*S*) and 2(*R*) orientations) to achieve the desired product.

Janes et al. disclose the use of α-chymotrypsin and bovine pancreatic protease for separating *cis* and *trans* diastereomers of 2(*R,S*)-benzyloxymethyl-1,3-dioxolane-4(*S*)-carboxylic acid methyl ester, wherein the hydrolysis occurs at the ester group. Janes et al. disclose that they discovered these two selective hydrolase enzymes "by screening a library of 91 commercial hydrolases." See the abstract. The *cis* and *trans* diastereomers of 2(*R,S*)-benzyloxymethyl-1,3-dioxolane-4(*S*)-carboxylic acid methyl ester are shown below:



cis



trans

In the initial screening test, the diastereoselectivity of the enzymes was estimated by determining their rates of hydrolysis with respect to the individual pure diastereomers, i.e., the *cis* 2(*S*)-benzyloxymethyl-1,3-dioxolane-4(*S*)-carboxylic acid methyl ester and the *trans* 2(*R*)-benzyloxymethyl-1,3-dioxolane-4(*S*)-carboxylic acid methyl ester. See Table 1. Based on these initial results, Janes et al. only selected 6 enzymes for further study, i.e., α-chymotrypsin, bovine pancreatic protease, substilisin from *Bacillus lichenformis*, bovine cholesterol esterase, protease

from *Streptomyces caespitosis*, and horse liver esterase. Diversa clonenzyme ESL-001-02 showed moderate estimated diastereoselectivity, but was not selected for further study because of its expense. See page 9021 right column.

The disclosure of Janes et al. actually teaches away from applicants' claimed invention. As noted above, of the 91 enzymes screened, Janes et al. selected only 6 enzymes as warranting further study. Included among the tested enzymes that exhibited insufficient "estimated diastereoselectivity" were pig liver esterase, Candida Antarctica "A" lipase, Candida lypolitica lipase, and Mucor miehei lipase. Additionally, it is noted that Janes et al. disclose that *Candida Antarctica* lipase B showed no detectable activity with respect to hydrolysis of the substrate. See footnote (*a*) of Table 1 at page 9023. Compare the enzymes recited in applicants' claims.

Additionally, at page 9021 of Janes et al. clearly state that "although 21 hydrolases favored the *cis*-dioxolane, their estimated diastereoselectivites were low, D<1.1-6.9." Based on this result, the authors decided to proceed with six trans selective hydrolases enzymes that had estimated diastereoselectivities greater than 8. Therefore, based on these results, the hydrolases having an estimated diastereoselectivity of less than 8 would not be of interest to a person of ordinary skill in the art seeking to selectively hydrolyze other dioxolane compounds.

See also the disclosure by Janes et al. at page 9023, right column, first paragraph. Here, Janes et al. acknowledged that the "quick D values for the six hydrolases were significantly lower, sometimes more than 20 times, than the estimated diastereoselectivities, Table 2." Thus, even for the selected 6 trans selective hydrolases enzymes, the second step in screening process (the quick D measurement) showed that the diastereoselectivity was lower than that suggested by the initial screening results. Thus, this would clearly lead one of ordinary skill in the art away from using the hydrolases that were found to be insufficient in the initial screening tests.

Further, as noted above, the substrate used by Janes et al., 2-(*R/S*)-benzyloxymethyl-4(*S*)-carboxylic acid-1,3-dioxolane methyl ester, has only one cleavable carbonyl group at the C-4 position. Since Janes et al. disclose that certain enzymes had insufficient activity with respect to this substrate with one cleavable carbonyl group, one of ordinary skill in the art would be discourage from using such enzymes on a substrate that had two cleavable carbonyl groups.

With regards to applicants' claim 12, the article by Janes et al. focuses on **trans** selective

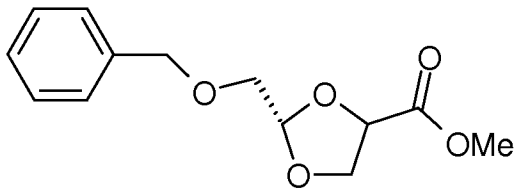
hydrolases enzymes. Based on the initial screening, no enzyme that favored the *cis*-dioxolane was selected for further research due to their low estimated diastereoselectivites. Janes et al. provide no suggestion of an enzymatic process wherein the *cis*-isomer of the diastereoisomer dioxolanes applicants' formula IV is selectively hydrolyzed, yielding the unhydrolyzed *trans*-isomer dioxolane compound of applicants' Formula III. Compare applicants' claim 12.

Cimpoia et al. (WO '759) disclose a process for separating ß and α anomers from an anomeric mixture. The anomeric mixture is hydrolyzed with an enzyme selected from cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from *Streptomyces caespitosis*, substilisin from *Bacillus lichenformis*, protease from *Aspergillus oryzae*, proteinase from *Bacillus lichenformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus mellus*, proteinase from *Bacillus substilis*, ESL-001-05, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, lipase from *Pseudomonas cepacia,* and bacterial proteinase. See page 7, line 6 - page 8, line 7.

Cimpoia et al. (WO '759) do not disclose use of the enzymes Pig Liver Esterase or Porcine Pancreatic Lipase enzyme. Compare applicants' claim 1. In addition, Cimpoia et al. (WO '759) also fail to disclose the use of Candida Antarctica "A" lipase, Candida Antarctica "B" lipase, Candida Lypolitica Lipase, or Rhizomucor Miehei Lipase. Compare applicants' claim 12.

Additionally, like Janes et al., Cimpoia et al. (WO '759) fails to suggest the use of such enzymes on a substrate recited in applicants' claims. In particular, the disclosure of Cimpoia et al. (WO '759) does not suggest to one of ordinary skill in the art the use of, for example, Pig Liver Esterase enzyme, Porcine Pancreatic Lipase enzyme, Candida Antarctica "A" lipase, Candida Antarctica "B" lipase, Candida Lypolitica Lipase, or Rhizomucor Miehei Lipase for resolution of a 2,4 disubstituted dioxolane having two cleavable carbonyl groups.

In Example 1 of Cimpoia et al. (WO '759), an anomeric mixture of the compound 2-(S)-**benzyloxy**methyl-4-carboxylic acid-1,3-dioxolane methyl ester is subjected to hydrolysis using α-Chymotrypsin. This substrate compound has the following formula:



In this isomeric mixture of 2-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester the orientation at the 2-position is fixed, i.e., 2-(*S*). Thus, the mixture contains isomers that differ with respect to the orientation at the 4-position. By comparison, in the isomers of 2-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester tested by Janes et al., the orientation at the 4-position is fixed, i.e., 4-(*S*), while the orientation at the 2-position differs.

In any event, in the compound of Example 1 of Cimpoia et al. (WO '759), the C-2 group is a benzyloxymethylene group and the C-4 group is a cleavable carbonyl group, i.e., -CO-O-Methyl. Conversely, the compounds of applicants' formula II have a cleavable carbonyl group at both the C-2 and C-4 positions, i.e., the -O-R2/R12 and -CO-O-R1/R11 groups, respectively. See the definition of R1/R11 and R2/R12 in claims 1 and 12.

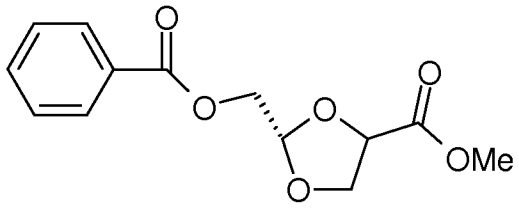
Example 1 of Cimpoia et al. (WO '759) teaches the use of chymotrypsin to resolve 4(S)/4(R) isomers having one cleavable carbonyl group at the C-4 position, i.e., the ester group. Compare the disclosure of Janes et al. wherein α-chymotrypsin is used to separate *cis* and *trans* diastereomers of 2(*R,S*)-benzyloxymethyl-1,3-dioxolane-4(*S*)-carboxylic acid methyl ester, wherein the hydrolysis also occurs at the ester group

Example 1 of Cimpoia et al. (WO '759) does not provide any suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups, i.e., at the C-2 and C-4 position. Such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e., discriminating between the 2(S) and 2(R) orientations) to achieve the desired product.

Examples 28, 30, 32, 34, 36, and 38 of Cimpoia et al. (WO '759) are similar to Example 1 in that they involve separating a 2:1(β:α) anomeric mixture of the compound 2-(S)-benzyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester, but use different enzymes then that of Example 1. But, here again, these Examples provide no suggestion as to what enzyme, if any, would be effective, i.e., both regioselectively and diastereoslectively, for the separation of

2(S) and 2(R) isomers having two carbonyl cleavable groups.

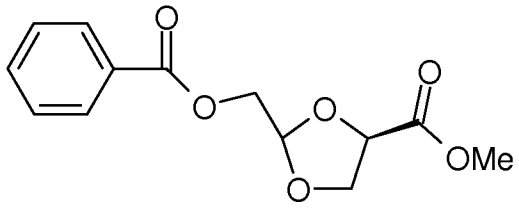
In Example 4 of Cimpoia et al. (WO '759), a 2:1(β:α) anomeric mixture of the compound 2-(S)-benzoyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester is subjected to hydrolysis using protease from *Aspergillus oryzae*. This substrate compound has the following formula:



Thus, in this compound the C-2 and C-4 groups are both group cleavable carbonyl groups, i.e., benzoyloxymethylene and -CO-O-Methyl, respectively. However, this Example provides no suggestion of the use of the enzymes recited in applicants' claims. Additionally, in this Example the orientation at the 2-position is fixed. Thus, the Example does not involve the separation of 2(S) and 2(R) isomers having two carbonyl cleavable groups

Examples 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, are similar to Example 4 in that they involve separating a 2:1(β:α) anomeric mixture of the compound 2-(S)-benzoyloxymethyl-4-carboxylic acid-1,3-dioxolane methyl ester, but use different enzymes then that of Example 4, i.e., proteinase from *Bacillus lichenformis*, substilisin from *Bacillus lichenformis*, protease from *Streptomyces griseus*, acylase from *Aspergillus mellus*, proteinase from *Bacillus substilis*, ESL-001-02, pronase protease from *Streptomyces griseus*, lipase from *Rhizopus arrhizus*, lipoprotein lipase from *Pseudomonas* species type B, bacterial proteinase, and lipase from *Pseudomonas cepaciaI.* But, here again, these Examples provide no suggestion of the use of the enzymes recited in applicants' claims. Also, in these Examples the orientation at the 2-position is fixed.

In Example 47 of Cimpoia et al. (WO '759), an anomeric mixture of the compound 2-benzoyloxymethyl-4(R)-carboxylic acid-1,3-dioxolane methyl ester is subjected to hydrolysis using α-Chymotrypsin. This substrate compound has the following formula:



Thus, in this compound the C-2 and C-4 groups are both cleavable carbonyl groups, i.e., benzoyloxymethylene and -CO-O-Methyl, respectively. Thus, this Example involves treating a mixture 2(S) and 2(R) isomers, while retaining the 4(R) configuration the same, to obtain the cis 2(R), 4(R) isomer. Conversely, the process of applicants' claims 1 and 12 are directed to enzymatic diastereomeric resolution of the 2(S) and 2(R) isomers while retaining the 4(S) configuration the same. In any event, Example 47 of Cimpoia et al. (WO '759) provides no suggestion of the use of the enzymes recited in applicants' claims.

For the reasons discussed above, Cimpoia et al. (WO '759) provides no rationale for modifying the disclosure of Janes et al. so as to arrive at a process in accordance with applicants' claim 1 or claim 12.

The rejection asserts that the article by Ferrero et al. discloses that "enzyme-catalyzed reactions have become standard procedures for the synthesis of enantiomerically pure compounds due to their simple feasibility and high efficiency." See page 586 of Ferrero et al. However, this statement merely acknowledges that the use of enzyme-catalyzed reactions in enantioselective synthesis procedures is known. This general statement by Ferrero et al. adds nothing to the disclosures of Janes et al. and Cimpoia et al. (WO '759) which already demonstrate the use of enzymes. But, moreover, the statement provides no suggestion for modifying the substrate used in the process of Janes et al. or the types of enzymes used in the processes of Janes et al. and Cimpoia et al.

The rejection further states that Ferrero et al. list in Table 1 commonly used enzymes and that this list includes pig liver esterase (PLE), porcine liver esterase (PPL), and Candida Antarctica "B" lipase (CAL). However, the inclusion of these enzymes in this general lists provides no suggestion as to the types or reactions that would use such enzymes, or the types or substrates that could be treated using such enzymes.

In the article by Ferrero et al., reaction procedures using PLE and PPL are illustrated in schemes 12 and 13 (pages 593-594), respectively. In these reactions, the substrates are tri-O-acylated nucleoside analogues having nucleosides bases (or derivatives thereof) attached to the C-4 position of the sugar ring. See also the use of PLE on di-O-acylated nucleoside analogues in scheme 15 (page 595). Thus, the substrate here does not have a cleavable carbonyl group at the

4-position of the sugar ring. Compare the substrates used in Janes et al. and in the Examples of Cimpoia et al. (WO '759).

Reactions schemes 12 and 13 also provide no suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups at the C-2 and C-4 position. As noted above, such an enzyme would need to act regioselectively (i.e., discriminating between the C-2 and C-4 positions) as well as diastereoslectively (i.e., discriminating between the 2(S) and 2(R)positions) to achieve the desired product.

In schemes 20-25 (pages 598-604), the substrates are nucleoside analogues having nucleosides bases (or derivatives thereof) attached to the C-4 position of the sugar ring. Thus, here also, the substrate here does not have a cleavable carbonyl group at the 4-position of the sugar ring. Compare again the substrates used in Janes et al. and in the Examples of Cimpoia et al. (WO '759).

In schemes 20-25, CAL is used to induce acylation or alkoxycarbonylation of the group at the C2 position of the sugar, not hydrolysis of the group attached to the C4 position of the sugar (i.e., the nucleoside base). Scheme 22 also shows the use of CAL to induce hydrolysis of an acyl group at the C2 position of the sugar. Thus, the disclosure of Ferrero et al. provides no suggestion of what enzyme would be useful to resolve 2(S)/2(R) isomers having two cleavable carbonyl groups at the C-2 and C-4 position. Moreover, the disclosure of Ferrero et al. provides no suggestion of using liver esterase (PLE), porcine liver esterase (PPL), or Candida Antarctica "B" lipase (CAL) in accordance with applicants' claimed invention.

For the reasons discussed above, Ferrero et al. provides no rationale for modifying the disclosure of Janes et al. or the disclosure of Cimpoia et al. (WO '759) so as to arrive at a process in accordance with applicants' claim 1 or claim 12.

Finally, the rejection relies on the disclosure of Martinelle et al. to assert that it would be obvious to use *Candida Antarctica* lipase B for resolution of a methylester. The rejection asserts that Martinelle et al. disclose that *Candida Antarctica* lipase B is "more typical of an esterase than a lipase." Applicants' disagree.

The comments by Martinelle et al. relate to *Candida Antarctica* lipase B showing a lack of "interfacial activation," not whether this lipase functions as an esterase. See also the Title of

the article, i.e., "On the interfacial activation of *Candida antarctica* lipase A and B as compared with *Humicola lanuginose* lipase." The portion of the disclosure of Martinelle et al. cited in the rejection reads as follows:

> "Our results for *Candida antarctica* lipase B (CALB) did not display any **interfacial activation** towards PNPA and PNB in the presence of an interface. The catalytic behavior of CALB was more typical of an esterase than of a lipase such as *Humicola lanuginose* lipase that showed **unambiguous interfacial activation** with the same substrates (Fig. 1, Fig.2 and Table 1)." (emphasis added)

See also the Introduction of the article by Martinelle et al. which discusses the differences in interfacial activation for esterases and lipases.

> "Triacylglycerol lipases (EC 3.1.1.3) are enzymes that catalyse the hydrolysis of neutral lipids in biological systems. A characteristic feature of triacylglycerol lipases is their **activation at water/lipid interfaces**. In contrast to esterases, lipases display almost no activity with their substrate in its monomeric state. It was suggested that **interfacial activation** of lipases is due to a conformational change in the protein leading to increased activity." (emphasis added).

Thus, the disclosure of Martinelle et al. adds nothing to the disclosures of Janes et al., Cimpoia et al., and Ferrero et al.

In view of the above remarks, one of ordinary skill in the art, taking the combined disclosures of Janes et al., Cimpoia et al., Ferrero et al., and Martinelle et al. would not be lead to modify the process of Janes et al. in such a manner as to arrive at a process in accordance with applicants' claimed invention. Thus, it is respectfully submitted that the disclosure of Janes et al., taken alone or in combination with the disclosure of Cimpoia et al., Ferrero et al., and/or Martinelle et al. fails to render obvious applicants' claimed invention. Withdrawal of the rejection is respectfully requested.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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